



Designation: D 3326 – 90 (Reapproved 2002)

Standard Practice for Preparation of Samples for Identification of Waterborne Oils¹

This standard is issued under the fixed designation D 3326; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers the preparation for analysis of waterborne oils recovered from water. The identification is based upon the comparison of physical and chemical characteristics of the waterborne oils with oils from suspect sources. These oils may be of petroleum or vegetable/animal origin, or both. Seven procedures are given as follows:

	Sections
Procedure A (for samples of more than 50-mL volume containing significant quantities of hydrocarbons with boiling points above 280°C)	8 to 12
Procedure B (for samples containing significant quantities of hydrocarbons with boiling points above 280°C)	13 to 17
Procedure C (for waterborne oils containing significant amounts of components boiling below 280°C and to mixtures of these and higher boiling components)	18 to 22
Procedure D (for samples containing both petroleum and vegetable/animal derived oils)	23 to 27
Procedure E (for samples of light crudes and medium distillate fuels)	28 to 34
Procedure F (for thin films of oil-on-water)	35 to 39
Procedure G (for oil-soaked samples)	40 to 44

1.2 Procedures for the analytical examination of the waterborne oil samples are described in Practice D 3415 and Test Methods D 3327, D 3328, D 3414, and D 3650. Refer to the individual oil identification test methods for the sample preparation method of choice. The deasphalting effects of the sample preparation method should be considered in selecting the best methods.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific caution statements are given in Sections 6 and 32.

2. Referenced Documents

2.1 ASTM Standards:

¹ This practice is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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- D 95 Test Method for Water in Petroleum Products and Bituminous Materials by Distillation²
- D 96 Test Method for Water and Sediment in Crude Oil by Centrifuge Method (Field Procedure)²
- D 1129 Terminology Relating to Water³
- D 1193 Specification for Reagent Water³
- D 1959 Test Method for Iodine Value of Drying Oils and Fatty Acids⁴
- D 1983 Test Method for Fatty Acid Composition by Gas-Liquid Chromatography of Methyl Esters⁴
- D 2800 Test Method for Preparation of Methyl Esters from Oils for Determination of Fatty Acid Composition by Gas Chromatography⁴
- D 3325 Practice for Preservation of Waterborne Oil Samples⁵
- D 3327 Test Methods for Analysis of Selected Elements in Waterborne Oils⁶
- D 3328 Test Methods for Comparison of Waterborne Petroleum Oils by Gas Chromatography⁵
- D 3414 Test Method for Comparison of Waterborne Petroleum Oils by Infrared Spectroscopy⁵
- D 3415 Practice for Identification of Waterborne Oils⁵
- D 3650 Test Method for Comparison of Waterborne Petroleum Oils by Fluorescence Analysis⁵
- D 4489 Practices for Sampling of Waterborne Oils⁵
- E 1 Specification for ASTM Thermometers⁷
- E 133 Specification for Distillation Equipment⁸

3. Terminology

3.1 *Definitions*—For definitions of terms used in this practice, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

² Annual Book of ASTM Standards, Vol 05.01.

³ Annual Book of ASTM Standards, Vol 11.01.

⁴ Annual Book of ASTM Standards, Vol 06.03.

⁵ Annual Book of ASTM Standards, Vol 11.02.

⁶ Discontinued; see 1993 Annual Book of ASTM Standards, Vol 11.02.

⁷ Annual Book of ASTM Standards, Vol 14.03.

⁸ Annual Book of ASTM Standards, Vol 14.04.

3.2.1 *animal/vegetable-derived oils*—a mixture made of mono-, di-, and triglyceride esters of fatty acids and other substances of animal or vegetable origin, or both.

3.2.2 *Simulated weathering of waterborne oils by distillation* considers only the effect of evaporation, which likely is the most significant short-term weathering effect in the environment.

3.2.3 *Simulated weathering of waterborne oils by evaporation* under ultraviolet light simulates the loss of light components on weathering, as well as some oxidative weathering.

4. Significance and Use

4.1 Identification of a recovered oil is determined by comparison with known oils selected because of their possible relationship to the particular recovered oil, for example, suspected or questioned sources. Thus, samples of such known oils must be collected and submitted along with the unknown for analysis. It is unlikely that identification of the sources of an unknown oil by itself can be made without direct matching, that is, solely with a library of analyses.

5. Reagents and Materials

5.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.⁹ Special ancillary procedures such as fluorescence may require higher purity grades of solvents. Other grades may be used provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent Type II water conforming to Specification **D 1193**.

6. Caution

6.1 Solvents used in this practice are volatile, flammable, or may cause the harm to the health of the user. Specifically, benzene is a known carcinogen, while chloroform and carbon tetrachloride are suspected carcinogens. Consequently, it is important that extractions and separations utilizing these substances must be carried out in a laboratory hood with a minimum linear face velocity of 38 to 45 m/min (125 to 150 ft/min) located in a regulated area posted with signs bearing the legends: NO SMOKING or (if appropriate) DANGER-CHEMICAL CARCINOGEN-AUTHORIZED PERSONNEL ONLY, or both.

7. Sampling

7.1 Collect representative samples in accordance with Practices **D 4489**.

7.2 Preserve the waterborne oil samples in accordance with Practice **D 3325**.

7.3 The portion of the sample used must be representative of the total sample. If the material is liquid, thoroughly stir the sample as received, warming if necessary to ensure uniformity.

PROCEDURE A—LARGE SAMPLES

8. Scope

8.1 This procedure covers the preparation for analysis of samples in which the volumes of waterborne oil in the environmental and suspect source samples equal or exceed 50 mL and in which the oil portion contains significant amounts of hydrocarbons with boiling points above 280°C.

NOTE 1—The boiling point may be ascertained by injecting the neat samples into the gas chromatograph and checking the elution times above that of pentadecane on a nonpolar column.

8.2 The preparation of samples containing mostly hydrocarbons of boiling points below 280°C, such as petroleum distillate fuels, is beyond the scope of this procedure (see Procedure C or E).

9. Summary of Procedure

9.1 A neat portion of the waterborne oil is retained. If not possible to obtain a neat portion, then retain a portion of the waterborne oil as received. This is to be used in those analyses performed on samples containing significant quantities of hydrocarbons with boiling points below 280°C. Preparation of these samples is beyond the scope of this procedure, but are covered in Procedure C.

NOTE 2—Waterborne oil samples containing significant quantities of hydrocarbons with boiling points below 280°C (see **Note 1**), such as gasoline and kerosene, can usually be obtained as neat samples without any sample preparation.

9.2 The waterborne oil sample is dissolved in an equal volume of chloroform or dichloromethane and centrifuged to remove the free water, solids, and debris in accordance with Test Method **D 96**. The water layer, if present, is separated from the organic layer. Other debris, if present, is removed by filtration through glass wool.

NOTE 3—The use of spectrograde cyclohexane is required for the extraction of samples to be analyzed by fluorescence spectrometry by Test Method **D 3650**. Separation of water may be accomplished by centrifugation or drying, or both, with anhydrous sodium sulfate.

9.3 When centrifugation will not separate the water from the chloroform solution of the sample, it is refluxed with an aromatic or petroleum distillate solvent in accordance with Test Method **D 95**.

NOTE 4—Pressure filtration has also been found useful for breaking emulsions.

9.4 A portion of the solvent/sample solution is retained. The solvent may be removed by evaporation. This portion of the sample may be used in the preliminary gas chromatographic analysis, Test Methods **D 3328** (Test Method A), and other analyses in which the results are unaffected by weathering.

9.5 The remainder of the solvent/sample solution is distilled using nitrogen purge to a liquid temperature of 280°C to

⁹ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

remove the solvent and simulate weathering conditions as nearly as possible. The distillate may be discarded or saved for characterization by gas chromatography (Test Methods D 3328). This simulated weathering treatment is necessary to bring the unweathered suspect samples and the waterborne oil sample to as nearly comparable physical condition for subsequent analysis as possible. Analyses requiring the use of this treated residue include elemental analysis (Test Methods D 3327); gas chromatographic analysis (Test Methods D 3328, Test Methods A and B); an infrared procedure (Test Method D 3414); a fluorescence test method (Test Method D 3650); and any applicable test method or practice described in Practice D 3415.

NOTE 5—The distillate might yield useful information but is discarded in this practice.

10. Apparatus

10.1 *Centrifuge*, capable of whirling two or more filled 100-mL centrifuge tubes at a speed that is controlled to give a relative centrifugal force (rcf) between 500 and 800 at the tip of the tubes, as specified in Test Method D 96.

10.2 *Centrifuge Tubes*, cone shaped, 100 mL, as specified in Test Method D 96.

10.3 *Distillation Apparatus for Water Determination*, as specified in Test Method D 95.

10.4 *Distillation Apparatus for Simulated Weathering*, as described in Specification E 133 except fitted with nitrogen-stripping tubulation as illustrated in Fig. 1.

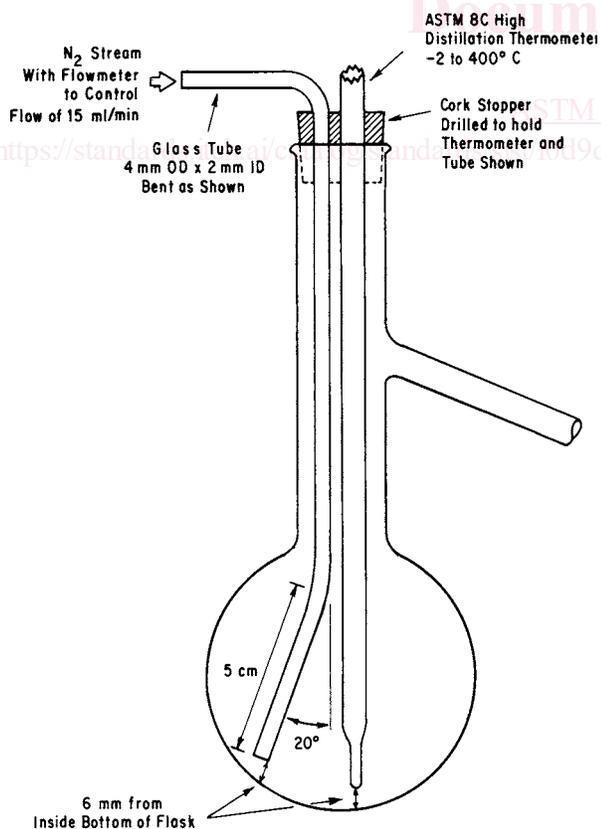


FIG. 1 Adaptation of ASTM Distillation Flask for Topping Chloroform Solutions of Oil to Simulate Weathering

10.5 *Distillation Flask*, 200 mL, as described in Specification E 133.

10.6 *Thermometer*, ASTM high distillation, having a range from -2 to +400°C and conforming to the requirements for thermometer 8C as prescribed in Specification E 1.

10.7 *Flowmeter*, to regulate flow of nitrogen to distillation flask. It should be calibrated and graduated for the range 10 to 15 mL/min.

11. Reagents and Materials

11.1 *Filter Paper*, medium retention, medium fast speed, prewashed with solvent used.

11.2 *Glass Wool*, prewashed with solvent used.

11.3 *Solvent*—Chloroform (stabilized with ethanol) or dichloromethane is used for dissolution of the waterborne oil samples. If water is to be removed by distillation, an aromatic, petroleum distillate, or volatile spirits solvent is required as specified in Test Method D 95. The safety precautions associated with the use of the solvent selected should be considered before it is used (see Note 3).

12. Procedure

12.1 *Retention of Neat Samples:*

12.1.1 Decant or siphon off a portion of the neat waterborne oil if possible.

12.1.2 If not possible to obtain a neat sample, retain a portion of the original oil.

12.2 *Removal of Water, Sediment, and Debris:*

12.2.1 Transfer about 50 mL of original waterborne oil to a 100-mL centrifuge tube. Add about 50 mL of chloroform or dichloromethane to the tube and mix thoroughly. For waxy samples, use chloroform. Warm solutions to 50°C to prevent precipitation (see Note 3).

12.2.1.1 Centrifuge the mixture at 500 to 800 rcf (relative centrifugal force) for 10 min to separate free water and solids as specified in Test Method D 96. For waxy samples, use chloroform. Warm solutions to 50°C to prevent precipitation (see Note 3).

12.2.1.2 Withdraw the water layer if present. Decant the chloroform or dichloromethane solution to a sample bottle. Filter through a glass wool plug, if necessary, to afford a clean separation.

12.2.2 Process those samples from which water cannot be separated by centrifugation by Test Method D 95 distillation procedure. Filter the dry solution through medium retention filter paper. Rinse filter paper with solvent to remove oil. For waxy samples, use chloroform and keep filter funnel and contents at 50°C during filtration (see Note 3).

12.2.3 Starting at 12.1, treat all reference or suspect samples in an identical fashion. If it is apparent that the reference or suspect samples contain less than 1% water and sediment, centrifugation may be eliminated and the reference or suspect samples should be diluted with an equal volume of chloroform or dichloromethane before proceeding.

12.3 *Removal of Solvent and Simulated Weathering:*

12.3.1 Transfer approximately 100 mL of the solution to a chemically clean 200-mL flask. Assemble apparatus so the ASTM high distillation thermometer (8C) and nitrogen stripping tubulation are about 6 mm from the bottom of the flask.

Direct flow away from thermometer bulb to prevent local cooling of thermometer (see Fig. 1).

12.3.2 Perform distillation using a nitrogen flow of 10 to 15 mL/min. Terminate distillation at a liquid temperature of 280°C. Shut off the nitrogen flow when the temperature of the liquid in the distillation flask cools below 175°C. Pour the hot residue into a suitable container.

12.3.3 Treat all reference and suspect oils in the same manner as the waterborne oil samples. Repeat 12.2.1-12.3.2.

PROCEDURE B—LIMITED SAMPLE VOLUMES OF HEAVY OILS

13. Scope

13.1 This procedure covers the preparation for analysis of waterborne oil samples of petroleum derived origin in which the volumes equal or are less than 1 mL. An aliquot of larger oil samples may also be used.

13.2 The procedure is applicable to oils containing significant amounts of hydrocarbons boiling above 280°C.

13.3 The preparation of samples containing lower boiling hydrocarbon is beyond the scope of this procedure, but is covered by Procedures C and E.

14. Summary of Procedure

14.1 The sample is dissolved in pentane or hexane, and the water and insolubles are removed by centrifugation. The organic solvent phase is dried with anhydrous magnesium sulfate, filtered, and the volatile components and solvents are removed by evaporation under a nitrogen stream (see Note 3).

15. Apparatus

15.1 *Centrifuge*, see 10.1.

15.2 *Centrifuge Tubes*, see 10.2.

15.3 *Flow Control on Nitrogen Cylinder*, to control nitrogen flow over sample surface.

15.4 *Steam Bath*, or commercial temperature controlled solvent evaporator, maintained between 40 and 50°C.

16. Reagents and Materials

16.1 *Magnesium Sulfate*, anhydrous.

16.2 *Nitrogen*, a high purity grade.

16.3 *Pentane or Hexane*, chromatographic grade.

17. Procedure

17.1 Remove approximately 1 mL of the oil phase from the water-oil sample if possible and place it in a 100-mL centrifuge tube.

17.1.1 Add 40 mL of pentane or hexane and 1 g of anhydrous magnesium sulfate. Mix to remove water. If the sample tube is warm, additional magnesium sulfate may be required. Add magnesium sulfate in 1-g aliquots, mixing after each addition until no temperature change is detectable to the touch.

17.2 Alternatively, estimate the volume of oil in the sample and add approximately 40 vol of pentane per 1 vol of oil.

17.2.1 Shake or rapidly mix the oil and solvent.

17.2.2 Allow phases to separate, withdraw the solvent phase with a pipet, and place it in a 100-mL centrifuge tube.

17.3 Centrifuge as described in 12.2.1.1 for 5 min.

17.4 Decant supernatant liquid into a 250-mL beaker and evaporate the solvent and volatiles initially at 25 to 35°C and then at 40 to 50°C for 2 h in the presence of a stream of nitrogen. Transfer the sample to a sample vial when there is approximately 4 mL sample remaining and continue the solvent removal. The samples can then be used for analysis in accordance with Practice D 3415.

NOTE 6—This treatment with 70 mg of oil, evaporated at 40°C for 15 min in the presence of an airstream, yielded gas chromatograms resembling those of the distillation test method in 12.3.¹⁰

PROCEDURE C—OILS BOILING BELOW 280°C

18. Scope

18.1 This procedure covers the preparation for analysis of waterborne oil samples containing significant amounts of components boiling below 280°C.

18.2 The procedure is applicable to samples of distillate fuel oils, light and heavy naphthas, and other petroleum solvents.

19. Summary of Procedure

19.1 The oil and water phases are separated by centrifugation, and the oil phase is dried with anhydrous magnesium sulfate.

20. Apparatus

20.1 *Centrifuge*, see 10.1.

20.2 *Centrifuge Tubes*, see 10.2.

20.3 *Separatory Funnel*, glove or pearshaped, 100 mL, with TFE-fluorocarbon stopcock.

20.4 *Pipets*, disposable glass.

21. Reagents and Materials

21.1 *Magnesium Sulfate*, anhydrous.

22. Procedure

22.1 Transfer up to 10 mL of sample into a 100-mL separatory funnel. If phases separate, withdraw and discard aqueous (lower) phase. Transfer the organic phase into a 12.5-mL centrifuge tube. Alternatively, if there is enough oil on the water, the oil may be transferred directly with a pipet. Proceed to 22.4.

22.2 Prepare emulsified samples in the following manner: Transfer 10 mL of the sample to a centrifuge tube and centrifuge for 30 min at 1000 rcf (relative centrifugal force). If an oil layer appears, remove and proceed as directed in 22.4.

22.3 If a distinct oil layer does not appear, add to the test tube a maximum of 1 g of sodium chloride, mix, and centrifuge as in 22.2. If separation does not occur after centrifugation, add pentane, hexane, or cyclohexane, up to one quarter the sample volume, mix thoroughly, and proceed as in Procedure B.

22.4 Add 1 g of anhydrous magnesium sulfate, and mix for 1 min. If the sample tube is warm, additional magnesium

¹⁰ Gruenfeld, M., and Frederick, R., "The Ultrasonic Dispersion, Source Identification, and Quantitative Analysis of Petroleum Oils in Water," Rapp. P-V, Reun. Cons. int. Explor. Mer. 171:33, 1977.